Genetic Susceptibility, Residential Radon, and Lung Cancer in a Radon Prone Area

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Introduction: Radon exposure has been classified as the second cause of lung cancer, after tobacco, and the first in never smokers. *GSTM1* and *GSTT1* genes deletion increase the risk of lung cancer. We aim to know whether the risk of lung cancer because of residential radon is modulated by these genetic polymorphisms.

Methods: Hospital-based, case-control study where cases had confirmed lung cancer. Cases and controls did not have previous neoplasm and were older than 30. Controls attended hospital for noncomplex surgery. We analyzed the results for the whole sample and separately for never/light smokers and moderate/heavy smokers.

Results: Seven-hundred and ninety-two participants were analyzed. GSTM1 and GSTT1 deletion conferred an odds ratio (OR) of 1.38 (95% confidence interval [CI] 0.93–2.04) and 1.13 (95% CI 0.70–1.82), respectively. Individuals with GSTM1 present and residential radon concentrations higher than 148 Bq/m^3 had an OR of 1.48 (95% CI 0.73–3.00), whereas those with GSTM1 deleted had an OR of 2.64 (95% CI 1.18–5.91) when compared with participants with GSTM1 present and radon concentrations below 50 Bq/m3. Similar results were observed for GSTT1 deletion. These results were basically the same for the moderate/heavy smokers' subgroup.

Conclusions: The absence of *GSTM1* and *GSTT1* genes increases the risk of lung cancer because of radon exposure. These genes might modulate the carcinogenic pathway of alpha radiation. Further studies are warranted analyzing this association in never smokers.

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Lung cancer is a major health problem in developed countries. It is the leading cause of cancer death in males and females in the United States, according to recent data. Lung cancer mortality in females doubles that caused by breast cancer.[1] Survival has hardly improved in the last 30 years, with a 13–17% 5-year survival rate.[1,2]

Lung cancer incidence in Spanish males has an intermediate position regarding other European Union countries. Standardized incidence rate (2012 estimations) was 66.3 cases/100,000 persons-year in Europe and 76.8 in Spain. Lung cancer incidence in females was one of the lowest (26.1 cases/100,000 persons-year in Europe versus 15.7 in Spain). Lung cancer is the second cause of mortality and the first cause of cancer death in Spanish males.[3]

Radon is the second cause of lung cancer after active smoking and the leading cause in never smokers.[4] The United States Environmental Protection Agency (USEPA)[5] and the World Health Organization[4] have published recommendations to aware citizens on radon exposure hazards.

Radon emits radiation in form of α-particles that damage lung epithelia by generating oxygen-anions and hydrogen that produce mutations and other DNA lesions.[6] Neighboring nonirradiated cells may also be damaged through a "bystander effect," whereby cellular signaling from an irradiated cell may induce oxidative stress in adjacent but nonirradiated cells.[7]

Genetic polymorphisms on genes participating in detoxification processes of environmental carcinogens can modulate lung cancer risk. Animal models suggest that several gene polymorphisms may cooperate in increasing the individual risk of lung cancer.[8]

Polycyclic aromatic hydrocarbons and aromatic amines are classes of compounds that cause cancer in humans. Microsomal epoxide hydrolase 1 (EPHX1) plays an important role in both the activation and detoxification of polycyclic aromatic hydrocarbons and aromatic amines. A meta-analysis has indicated that in white population, the high activity variant genotype of EPHX1 polymorphisms at exon 4 was associated with a modest increase in the risk of lung cancer.[9] Cytosolic glutathione S-transferase family are a large family

of isozymes involved in detoxification of many electrophilic substrates. The *mu* (GSTM1) and *theta* (GSTT1) members are susceptibility genes because of their ability to regulate the conjugation of carcinogenic compounds to excretable hydrophilic metabolites.[10] *GSTM1* and *GSTT1* genes are deleted among 50% and 20% of Caucasians, respectively,[11] and this results in the lack of an active enzyme.[10] Meta-analyses have indicated that the carriers of GSTM1 null or GSTT1 null genotypes have a higher risk of developing lung cancer compared with carriers of at least one functional allele.[12,13]

Despite the literature analyzing the effect of many genetic polymorphisms on the risk of lung cancer and their interaction with tobacco consumption, there are very few studies assessing residential radon exposure in combination with different variants of susceptibility genes. We have found only one case-only study[14] providing evidence of a GSTM1-radon interaction on the risk of lung cancer. There is also no information regarding whether the possible effect of radon exposure modulated by these genes could be different for never/light smokers or moderate/heavy smokers.

The aim of the present study is to assess, to our knowledge for the first time through a case-control study, whether there is any effect modification between polymorphisms in *GSTM1*, *GSTT1,* and *EPHX1* genes and residential radon exposure on the risk of lung cancer. A secondary objective is to assess whether this effect is different for never/light smokers or moderate/heavy smokers.

MATERIALS AND METHODS

Design, Subjects, and Settings

A hospital-based, case-control study was conducted in Northwest Spain (Galicia) between 2004 and 2008. Galicia is characterized for having high indoor radon concentrations because of the granitic nature of the earth crust.[15,16] Approximately, 19–21% of all dwellings are above the USEPA action level. This fact places Galician population on a natural experiment where research on radon-related health effects can take advantage. A further advantage is that previous studies have demonstrated that Galician population has low mobility. The median of years living in the same residence is 30,[17] and therefore radon effects can be attributed easier than in other populations.

We recruited cases and controls at two Galician hospitals with full capacity to diagnose and treat lung cancer. The participating hospitals were the Santiago de Compostela Clinic University Hospital and the Ourense Hospital Complex. Both had to be older than 30 and should have lived at least 5 years in their current residence. Individuals with previous cancer were excluded. Cases had an anatomopathologically confirmed lung cancer and were recruited through consecutive sampling. Cases were identified through checking at least twice per week the databases of the Pathologic Anatomy Department. They were interviewed immediately after diagnosis. Controls were recruited from individuals attending hospital for noncomplex surgery unrelated with tobacco consumption. We selected controls with this characteristic to avoid selection bias in order that controls can represent adequately tobacco consumption of the general population and not over represent it. Two days per week one researcher recruited controls from the presurgery unit of both participating hospitals that fulfilled the inclusion criteria. More than 90% of the controls underwent the following surgeries: orthopedic surgery, cataract surgery, or surgery for inguinal hernias. We did a frequency-based sampling of controls regarding cases on age and gender to guarantee that both were comparable for these two variables. The study protocol was approved by the Galician Clinical Research Ethics Committee (REF 2004/108) and informed written consent was obtained from all participants. The results of residential radon exposure on lung cancer have been published recently.[17]

Information Retrieval

All participants were interviewed by trained researchers using a detailed questionnaire with special attention on lifestyle habits. We collected detailed information regarding tobacco consumption. The questions on tobacco consumption were directed to the etiologic period, that is, 30 to 5 years previously to the time of the interview. For the purpose of this study, tobacco exposure was classified in four categories, never smokers, and smokers in tertiles according to their lifetime tobacco consumption: light smokers (1–33 packs/yr), moderate smokers (34–66 packs/yr) and heavy smokers (>66 packs/yr). We used these categories for adjusting the results by tobacco consumption.

Radon Measurements

A radon technician placed a detector in the participants' homes. The devices were of the alpha-track type (Radosys) and were away from doors, windows, or electrical devices and between 60 and 180 cm off the floor. The detectors were placed for a minimum of 6 months and radon concentrations were determined at the Galician Radon Laboratory at the Santiago de Compostela Clinic University Hospital. Seasonal adjustment was taken into account when the detectors were revealed. Quality controls are conducted periodically and our laboratory has been certified through intercomparison tests organized by the University of Cantabria with excellent results.[18]

Laboratory Methods

Total blood was collected in 5 ml EDTA tubes and was transferred to FTA classic cards (Whatman, Maidstone, United Kingdom). FTA cards were left to dry for a minimum of two hours and then stored in foil envelopes with a desiccant at room temperature. Before DNA extraction, a disc with a diameter of 2 mm was cut out from each of the FTA cards using a Harris Micro Punch tool and was placed into a clean Eppendorf. Discs were washed twice with FTA Purification Reagent and vortexed. Then, samples were washed twice in TRIS-EDTA (10 mM Tris, 0.1 mM EDTA, pH8) and were left to air dry for one hour.

Polymorphic deletion of the GSTM1 was determined by polymerase chain reaction (PCR) using β-interferon as an internal control. PCR conditions, including primer sequences were carried out following the protocol described previously by Khedhaier et al.[19] PCR products were amplified on the disc.

GSTs deletion and exon 4 EPHX single nucleotide polymorphisms SNP (rs2234922) was determined using MassARRAY SNP genotyping system (Sequenom Inc, San Diego, CA), according to manufacturer's instructions. The principles of this method are detailed in Buetow et al.[20] SNP genotyping services were provided by the Spanish "Centro Nacional de Genotipado" CeGen-ISCIII (www.cegen.org).

Statistical Analysis

A bivariate descriptive analysis was conducted to determine the distribution of the study variables according to the case or control status. Then, an unconditional logistic regression was performed, where the dependent variable was the case or control status and the independent variables were the presence or absence of the genes *GSTM1*, *GSTT1,* or *EPHX1* polymorphisms, and indoor radon exposure (divided into four categories: <50, 50–100, 101–147, and >147 Bq/m^3). As adjustment variables, we included age (continuous), gender, and tobacco consumption (in four categories). We performed the same analysis but stratifying the sample in never/light smokers (those who had smoked 10 or less pack-years in lifetime) and in moderate/heavy smokers (> 10 pack-years during lifetime). We did this analysis to assess possible differences on the effect of each gene due to tobacco consumption. We included light smoking cases with never smokers because there were only five light smokers.

To assess whether genetic polymorphisms modified the effect of residential radon exposure on lung cancer, we created three variables using syntaxes that combined simultaneously each gene variant with radon exposure. We therefore created a variable combining GSTM1 and radon exposure with six categories (GSTM1 present or null and radon exposure in 3 categories, being the reference category individuals with the gene present and exposed to <50 Bq/m^3). The same was done for *GSTT1* gene and radon exposure. For EPHX1, a variable with six categories was created (two for *EPHX1* gene, wild-type and G carrier (AG or GG) and three categories for radon exposure). We decided to join G carrier categories because there were only 29 individuals with the GG heterozygous form. The results of these models are presented adjusted by tobacco consumption, age, and gender. To explore the possibility of residual confounding because of tobacco consumption and to compare results for the whole sample and for moderate/heavy smokers, we did the same analysis only including them (>10 pack-years during lifetime). The results were adjusted by gender, age, and tobacco consumption. Because of the low number of never/light smoking cases (n = 39) this analysis was not done for this subgroup. We also assessed the possibility of an additive synergism between radon exposure (two categories with the cut point in 148 Bq/m3) and the genes assessed (classified as present, absent, or G carrier) using the method proposed by Hosmer and Lemeshow.[21] Results are expressed as odds ratios (OR) with their 95% confidence intervals. Analyses were conducted with SPSS version 17.

RESULTS

Nine-hundred and ninety individuals were initially included, 442 cases and 548 controls. The participation rates were high, 90% of all contacted cases and higher than 75% of all contacted controls. Eight-hundred and sixty-two participants (87%) of the 990 initially included, 349 (79.0%) cases and 513 (93.6%) controls, had complete radon measurements, but 70 individuals did not have a tobacco history allowing for the calculation of lifetime tobacco consumption, leaving 792 individuals. Of them, 716, 695, and 668 individuals had genotyping results for *GSTM1*, *GSTT1,* and *EPHX1* genes, respectively. Figure 1 displays the participation rate and the individuals available for the multivariate analysis.

Table 1 shows the sample description broken down by case and control status. Age and gender distribution among cases and controls was similar whereas tobacco consumption was higher for cases compared with controls. The tobacco categories shown in the table are the same used for the subgroup analysis that considers never/light smokers and moderate/heavy smokers, and it can be observed the low number of light smoking cases. There were 38 cases and 262 never/light smoking controls and 217 cases and 199 moderate/

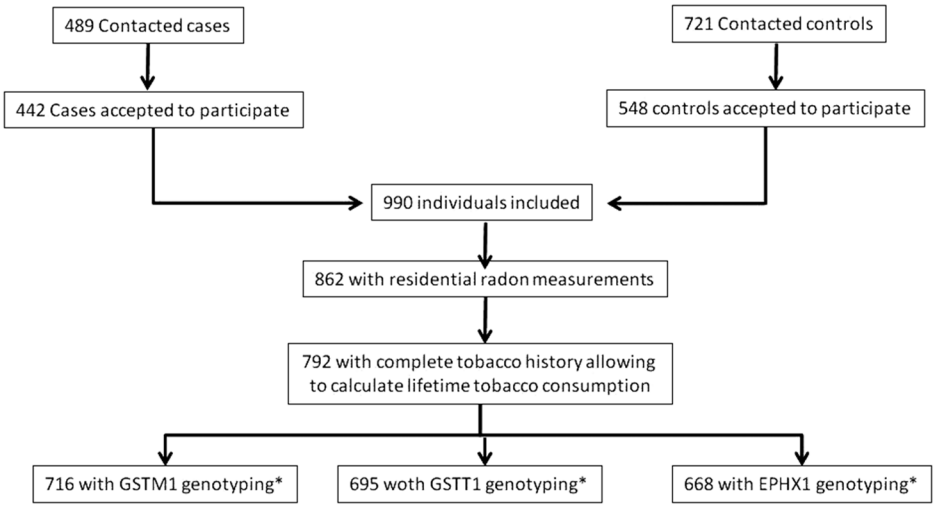

FIGURE 1. Flowchart describing participation and individuals included in the analysis. *Individuals included in the multivariate analysis and in the interaction models.

TABLE 1. Sample Description

Variables	Cases (n = 255) n (%)	Controls (n = 461) n (%)	p Value
Age			
≤50	16 (6.3)	43 (9.3)	> 0.05[a]
51–70	138 (54.1)	285 (61.8)	
>70	101 (39.6)	133 (28.9)	
Gender			
Female	34 (13.3)	59 (12.8	> 0.05[a]
Male	221 (86.7)	402 (87.2)	
Tobacco consumption			
Never smokers	33 (12.9)	209 (45.3)	<0.05[a]
Light smokers (1–10 pack-years)	5 (2.0)	53 (11.5)	
Moderate smokers (11–66 pack-years)	109 (42.7)	158 (44.3)	
Heavy smokers (>66 pack-years)	108 (42.4)	41 (8.9)	
Indoor radon exposure, Bq/m^3			
≤ 50	68 (26.7)	175 (38.0)	<0.05[a]
51–100	93 (36.5)	150 (32.5)	
101–147	43 (16.8)	67 (14.5)	
≥148	51 (20..0)	69 (15.0)	
Years living in the measured dwelling			>0.05[b]
Median	30	30	
Percentiles (25–75)	19–46	16–43	

[a]χ^2 test.
[b]t test.

heavy smoking controls. Indoor radon concentration was slightly higher for cases versus controls, especially in those exposed to concentrations higher than 148 Bq/m^3; 20% of cases versus 15% of controls surpassed 148 Bq/m^3 (USEPA action level).

Table 2 presents the effect of the absence of *GSTM1* and *GSTT1* genes and polymorphism in exon 4 of the *EPHX1* gene. GSTM1 is absent in 44.2% of cases versus 39.8% of controls. For the *GSTT1* gene, these figures are 21% for cases versus 19.2% in controls. The EPHX1 heterozygous was found in 33% of cases versus 34.8% of controls and the EPHX1 homozygous in 3.5% of cases versus 4.5% of controls. Although it seems to be no association with lung cancer for GSTT1 absence and EPHX1 exon 4 polymorphism, for the GSTM1 deletion, there is a marginally significant association, with an OR of 1.38 (95% CI 0.93–2.04). Results are adjusted by all the genes analyzed and also by age, gender, tobacco consumption, and residential radon concentration. When we analyze exclusively never and light smoking individuals, we can observe a higher risk of lung cancer when GSTM1 and GSTT1 are absent, with GSTT1 deletion having a marginally statistical effect. Heterozygous AG individuals for EPHX1 exon 4 polymorphisms show a protective effect (n = five AG cases). The results for moderate/heavy smokers are quite similar to the whole sample, specially for EPHX1 exon 4 polymorphisms. For GSTM1, we observe and OR of 1.15 (95% CI 0.75–1.75) and for GSTT1, the results are not statistically significant.

The effect on lung cancer risk of the different levels of residential radon combined with the presence or absence of *GSTM1* gene is showed in Table 3. It can be observed that for the same radon concentrations the risk of lung cancer is higher when GSTM1 is deleted and also that lung cancer risk increases when radon concentration increases. The OR for individuals exposed to indoor radon concentrations higher than 148 Bq/m^3 with GSTM1 present is 1.52 (95% CI 0.75–3.06) while for the same radon exposure when the gene is deleted is 2.64 (95% CI 1.18–5.91). No additive interaction was observed for GSTM1 deletion and radon exposure. When the analysis is restricted to moderate/heavy smokers, the results are similar to the whole sample. Lung cancer risks are higher for individuals lacking GSTM1 and exposed to higher radon concentrations than for individuals with the gene present exposed to similar radon levels.

TABLE 2. Risk of Lung Cancer by GSTM1, GSTT1, and EPHX1 Exon 4 Polymorphism

	Cases[a]	Controls[a]	Crude OR (95% CI)	Adjusted OR[b,c] (95% CI)	Adjusted OR[d] (95% CI) Restricted to Never and Light Smokers (≤ 10 Pack-Years Over Lifetime)	Adjusted OR[e] (95% CI) Restricted to Moderate/ Heavy Smokers (> 10 Pack-Years During Lifetime)
GSTM1	158	291	1 (—)	1 (—)	1 (—)	1 (—)
GSTM1 null	125	192	1.15 (0.83–1.59)	1.38 (0.93–2.04)	1.72 (0.73–4.03)	1.15 (0.75–1.75)
GSTT1	211	384	1 (—)	1 (—)	1 (—)	1 (—)
GSTT1 null	56	91	1.10 (0.74–1.63)	1.13 (0.70–1.82)	2.42 (0.91–6.45)	0.85 (0.51–1.42)
EPHX1 exon 4						
Wild-type (AA)	166	272	1 (—)	1 (—)	1 (—)	1 (—)
Heterozygous (AG)	86	156	0.91 (0.64–1.28)	0.83 (0.54–1.25)	0.33 (0.11–0.99)	0.91 (0.59–1.41)
Mutant (GG)	9	20	0.78 (0.35–1.76)	0.65 (0.24–1.79)	0.49 (0.05–5.02)	0.72 (0.25–2.05)

[a] The number of cases and controls is the used for the crude regressions.
[b]Adjusted for age, gender, tobacco consumption in four categories, indoor radon exposure in four categories and for all polymorphisms.
[c]The total number of individuals in the adjusted regression is 660: 233 cases and 427 controls.
[d]Includes 33 cases and 243 never smoking controls or who had smoked 10 pack-years or less in lifetime. Adjusted for age, gender, indoor radon exposure in four categories and for all polymorphisms.
[e]Includes 200 cases and 184 controls who smoked more than 10 pack-years in lifetime. Adjusted for age, gender, indoor radon exposure in four categories and for all polymorphisms.

TABLE 3. Residential Radon Exposure and GSTM1 Effect on Lung Cancer Risk

Indoor Radon Exposure (Bq/m3)	GSTM1 (Cases, Controls); OR[a,b] (95% CI)		Restricted to Moderate/Heavy Smokers (> 10 Pack-Years During Lifetime) GSTM1 (cases, controls); OR[a,c] (95% CI)	
	Present	Null	Present	Null
0–50	41, 95	27, 80	33, 44	23, 38
	1 (—)	0.87 (0.44–1.70)	1 (—)	0.95 (0.45–2.00)
51–147	77, 138	58,79	69, 55	48, 28
	1.39 (0.80–2.40)	2.32 (1.26–4.26)	1.74 (0.93–3.24)	2.70 (1.33–5.48)
>147	28, 46	24, 23	25, 21	19, 13
	1.52 (0.75–3.06)	2.64 (1.18–5.91)	2.16 (0.97–4.82)	2.41 (0.97–5.99)

[a]Adjusted for age, sex and tobacco consumption.
[b]Total sample (n = 716).
[c]n = 416.

Table 4 shows the effect of GSTT1 deletion for the different categories of residential radon exposure on the risk of lung cancer. The risk of lung cancer is higher when GSTT1 is absent for radon concentrations between 50 and 148 Bq/m^3. The odds ratio changes for radon exposures higher than 148 Bq/m^3. The OR for GSTT1 deleted is 1.10 (95% CI 0.39–3.08), whereas when the gene is present is 1.98 (95% CI 1.06–3.70). There were only eight cases and 16 controls with radon exposure higher than 148 Bq/m3 and GSTT1 absent. No additive interaction was observed between radon exposure and *GSTT1* gene. There are little differences with the former results when the analysis is restricted to moderate/heavy smokers.

Table 5 shows the effect of EPHX1 exon 4 polymorphism combined with different residential radon concentrations. The higher OR are observed for the wild-type when radon concentration is between 50 and 148 Bq/m^3, and for the G form (AG or GG) with the highest radon concentrations, with an OR of 3.05 (95% CI 1.25–7.43). No additive interaction was observed between radon exposure and EPHX1 exon 4 polymorphism. The results are essentially the same when the analysis is restricted to moderate/heavy smokers.

DISCUSSION

To our knowledge, this is the first study with a case-control design assessing multiple genetic polymorphisms in combination with residential radon exposure on the risk of lung cancer. We have observed that lung cancer risk for the same indoor radon concentration is higher when *GSTM1* or *GSTT1* genes are absent. No clear effect is evident for EPHX1 polymorphisms for different radon concentrations on the risk of lung cancer. When the analysis is restricted to moderate/heavy smokers (> 10 pack-years during lifetime) the results hardly differ when compared with the results of the whole sample. This gene-environment interaction is evident at radon concentrations below the action level recommended by the USEPA.

At first sight, it seems that the results observed for the analyzed genes could be driven by tobacco consumption. Table 2 shows that there are differences when never/light smokers and moderate/heavy smokers are analyzed separately. Nevertheless, for GSTM1 deletion, we observe a positive association for both categories, and the lowest confidence interval is very similar among them. We have to highlight that these results are adjusted by many covariables (gender, age, indoor radon exposure, and all studied genes) and that

TABLE 4. Residential Radon Exposure and GSTT1 Effect on Lung Cancer Risk

Indoor Radon Exposure (Bq/m^3)	GSTT1 (Cases, Controls); OR[a,b] (95% CI)		Restricted to Moderate/Heavy Smokers (> 10 Pack-Years During Lifetime) GSTT1 (Cases, Controls); OR[a] (95% CI)	
	Present	Null	Present	Null
0–50	55, 130	10, 43	48, 61	7, 19
	1 (—)	0.51 (0.21–1.22)	1 (—)	0.43 (0.16–1.19)
51–147	93, 183	35, 29	81, 69	30, 12
	1.34 (0.83–2.17)	3.70 (1.77–7.74)	1.53 (0.90–2.62)	3.00 (1.31–6.90)
>147	41, 52	8, 16	35, 24	6, 9
	1.98 (1.06–3.70)	1.10 (0.39–3.08)	2.22 (1.10–4.49)	0.96 (0.30–3.08)

[a]Adjusted for age, sex and tobacco consumption.
[b]Total sample (n = 695).
[c]n = 401.

TABLE 5. Residential Radon Exposure and EPHX1 Exon 4 Polymorphism Effect on Lung Cancer Risk

Indoor Radon Exposure (Bq/m³)	EPHX1 (Cases, Controls); OR[a,b] (95% CI)		Restricted to Moderate/Heavy Smokers (> 10 Pack-Years During Lifetime) EPHX1 (Cases, Controls); OR[a,c] (95% CI)	
	Wild-Type (AA)	G Carrier (AG and GG)	Wild-Type (AA)	G carrier (AG and GG)
0–50	40, 97	20, 68	33, 37	18, 38
	1 (—)	0.58 (0.28–1.21)	1 (—)	0.48 (0.21–1.06)
51–147	82, 116	45, 83	69, 45	42, 32
	1.90 (1.07–3.35)	1,27 (0.68–2.37)	1.66 (0.87–3.17)	1.59 (0.79–3.21)
>147	28, 49	21, 19	21, 24	20, 9
	1.26 (0.62–2.55)	3.05 (1.25–7.43)	1.02 (0.45–2.32)	3.44 (1.29–9. 17)

[a]Adjusted for age, sex, and tobacco consumption.
[b]Total sample (n = 668).
[c]n = 388.

there are only 33 never/light smoking cases included in this analysis. This fact produces wider confidence intervals for the category of never/light smokers than for ever-smokers and therefore imprecise results. A different effect for never/light and moderate/heavy smokers appears to be present for GSTT1 deletion, although in this case there are only 10 never/light smoking cases with the gene absent. These low numbers do not allow establishing a consistent conclusion regarding a different effect driven by tobacco consumption. The same explanation may apply to EPHX1 polymorphisms.

Regarding the possible differential effect of radon exposure on lung cancer risk depending on the presence or absence of the analyzed genes, it can be observed (Table 3) that: (1) lung cancer risk increases with radon exposure when GSTM1 is present or absent, (2) the risk appears to be higher when *GSTM1* gene is deleted, for all radon exposure categories and (3) the risk is quite similar for the whole sample and for moderate/heavy smokers. The same interpretation can be done for GSTT1 deletion and EPHX1 polymorphisms, although it is important to highlight the inconsistent results observed for GSTT1 null individuals exposed to the highest radon concentrations. There is no risk of lung cancer but this category includes only six cases and nine controls, a number much lower than the number of individuals existing for other categories. The following exposure category has 42 individuals, close to three times higher. A similar explanation could be given for EPHX1 wild-type individuals exposed to more than 147 Bq/m³, although in this case the number of participants is also low for G carriers. It is important to underline that the differences on lung cancer risk between wild-type and G carriers persist for those exposed to more than 147 Bq/m³.

A previous study from Bonner et al.[14] supports our observation for the GSMT1 null genotype. A plausible explanation is based on the knowledge that high-linear energy transfer α-particle, like those emitted by inhaled radon, causes DNA damage by the metabolic generation of reactive oxygen species (ROS),[6] which in turn, in the absence of regulatory enzymes such as glutathione S-transferase, induce signaling pathways that could preclude cancer appearance.[22]

Another important issue is the fact that the gene-environment interaction was mainly present at relatively low indoor radon concentrations. Taioli et al.[23] defined a low exposure-gene effect when a greater degree of gene-environment interaction appears at lower doses of exposure (the interaction follow an inverse dose function). Vineis et al.[24] also mention a exposure-gene effect, stating that there is a genetic heterogeneity, with a "depletion of susceptibles" at low dose levels, resulting in decreasing risk with increasing exposure. This possibility could explain the results observed for GSTT1 absence with high residential radon concentrations, opposite to expected.

A special case of interaction between radiation and gene polymorphisms is present in cytotoxicity caused by radiation therapy. When cells are exposed to ionizing radiation, decomposition occurs, through which a variety of ROS are generated.[25] Of all agents used, cyclophosphamide particularly results in generation of ROS, which was demonstrated by rodent data.[26] Under radiation therapy, individuals with GSTM1 null genotypes would experience a higher effective dose of chemotherapy and/or more reactive oxidant damage to tumor tissue. Therapy might then be more effective in GSTM1 null patients, as has been reported for GSTM1 null breast cancer patients.[27]

There is more evidence on the gene-environment interaction. Goto et al.[28] have proposed a model in which smokers with GSTM1 deletion would produce more carcinogen-DNA adducts in lung tissue and would develop mutations on genes involved in tumor growth and metastasis. Because of the presence of these mutations, GSTM1 null patients would present more aggressive tumor biology and poorer survival.[29]

Gamma radiation is another type of ionizing radiation, with a well described metabolism and genotoxicity. EPHX1 and GSTs play an important role in the .detoxification of electrophiles and oxidative stress. Vineis et al.[24] assessed the dose-dependent effect of genetic polymorphisms (EPHX, GSTP1, GSTM1, and GSTT1) in biotransforming on the induced genotoxicity by analyzing peripheral blood mononuclear cells from 20 individuals who were exposed to gamma radiation. They conclude that the influence of genetic polymorphisms of enzymes involved in DNA repair on induced genotoxicity depends on exposure dose.

Marcon et al.[30] carried out a mutagen sensitivity assay to investigate the role of inherited and acquired factors on

individual variation in DNA repair capacity. They irradiated fresh blood samples with gamma rays and detected spontaneous and induced structural chromosomal aberrations in lymphocytes 48 h after irradiation. They obtained a higher frequency of radiation-induced DNA aberrations in GSTM1 present individuals compared with GSTM1 null subjects. They concluded that a modulator effect of GSTM1 genotype on the individual DNA repair capacity is possibly related to the higher expression of enzymes involved in the repair of oxidative DNA damage in GSTM1 null subjects.

The relation between genotypes and chromosomic aberrations was also assessed in humans under irradiation. One study describes that the frequencies of chromosomic aberrations in a group of workers at Chernobyl Nuclear Station after the accident depend on some genotypes presented by participants.[31] Chromosomic aberrations in these workers were reduced in subjects with GSTM1 and GSTT1 null. However, the authors concluded that the elevated level of chromosomic aberrations is characteristic of an elevated level of spontaneous aberrations, and it does not completely correspond to genotypes.

Sakly et al.[32] assessed occupationally induced chromosomic damage in a large population of hospital workers exposed to low doses of ionizing radiation. GSTM1 and GSTT1 were postulated to be involved in the detoxification of endogenous and exogenous genotoxicants. They concluded that GSTM1 and GSTT1 polymorphisms do not modify significantly the genotoxic potential of ionizing radiation.

Despite the available evidence showing the implication of *GSTM1*, *GSTT1,* and *EPHX1* genes on the metabolic pathway on detoxification of ROS, the data are inconclusive. More biological support is needed to fully understand the interaction between ionizing radiation, genetic polymorphisms, and lung cancer risk because of residential radon exposure.

The study area is a radon prone region and this fact facilitates the analysis of a dose-response effect for radon exposure on lung cancer and also the interaction with susceptibility genes. In other settings, the analysis of interaction is not possible because of the homogeneity of radon exposure (low concentrations). Approximately 50% of the Galician population lives in the countryside in single detached houses. Because radon is denser than air, radon concentrations tend to be higher in isolated houses than in blocks of apartments. Cases and controls represent adequately the general population because there is universal health coverage provided by the Regional Government. Close to 100% of lung cancer cases are diagnosed in the public health system and the same happens for the surgical interventions performed in controls. A similar study but with a smaller sample size ($n = 319$) and not including radon measurements obtained similar results for the effect of GSTM1 and GSTT1 deletion on lung cancer risk.[33] This study included individuals with similar characteristics to those included in the present study.

The present study has important limitations. The sample size was not high enough to study in depth the effect of some polymorphisms. This was noted for the GSTT1 null and particularly for the EPHX1 exon 4 polymorphism as has been mentioned before. Prevalence for homozygous EPHX1 in cases was 3.4% versus 4.4% in controls, that are very close to the 3.2% prevalence of this polymorphism observed in Caucasians. Likewise, the prevalence for GSTT1 null in cases was 21% versus 19.2% in controls, that is very close to the 20% prevalence of this polymorphism in Caucasians.[11] Not all individuals had information available on radon exposure, tobacco consumption or genetic polymorphisms. Although this fact might increase the possibility of a selection bias, we think that this bias is not present. Individuals with data absent on those variables are quite similar to the individuals finally included in the analysis (data not shown). We have not been able to analyze properly the effect of susceptibility genes in never and light smokers (≤10 pack-years) and the possible differential effect for radon exposure in this subsample because of the low number of cases in this category ($n = 33$). Nevertheless, when we analyzed the possibility of a potential residual confounding because of tobacco consumption, we have observed that the results are very similar for moderate/heavy smokers compared with the results observed for the whole sample. A last limitation is that Galician population race is homogeneous (Caucasian whites) and therefore we cannot analyze the effect of these polymorphisms in different races.

Our study has also some advantages. The number of covariables taken into account in the analysis has been high. Results are adjusted by age, gender, tobacco consumption, three genetic polymorphisms, and also radon has been taken into account. The median number of years that cases and controls have been living in the same dwelling is very high, 30 years for both. Finally, all cases had a confirmed lung cancer diagnosis and were interviewed immediately after diagnosis to avoid recall bias. Controls were selected from nontobacco related surgery to minimize selection bias.

There is some possibility that the results observed could pose an impact. Many individuals could be interested in knowing whether they have a higher risk of lung cancer because of radon exposure related with the assessed polymorphisms. The existence of a possible effect modification of radon exposure conferred by susceptibility genes could increase radon awareness in population, especially in individuals living in radon prone areas.

To conclude, our study supports the hypothesis that the absence of *GSTM1* and *GSTT1* genes increases the risk of lung cancer in subjects exposed to residential radon, indicating that these genes can modulate the effect of environmental exposures. More studies focused exclusively in never smokers are warranted to know whether this effect persists in this subgroup of lung cancer cases. This research can provide some clues on the biology of radon-induced lung cancer, although the biological pathways should be studied taking into account more susceptibility genes along with genes participating in the regulation of the cell cycle.

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